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(54) Microencapsulation of water soluble polypeptides.

(57) This invention concerns novel sustained release microcapsule compositions comprising water-soluble, hormonally active polypeptides and optionally, a polymer hydrolysis modifying agent encapsulated in biocompatable, biodegradable polymers such as poly(lactide-co-glycolide) copolymers.

EP 0 052 510 A2

MICROENCAPSULATION OF WATER SOLUBLE POLYPEPTIDES

This invention relates to pharmaceutical microcapsule compositions having sustained release characteristics. The invention also relates to pharmaceutical compositions comprising a water-soluble polypeptide which is a luteinizing hormone/releasing hormone, or analogue thereof, useful for affecting the reproduction function in mammals.

There are a number of publications that disclose combinations of polymers and drugs designed to give sustained or delayed release of drugs. For example U.S. Patent 3,773,919 discloses controlled drug release compositions in which the core comprises a drug, stated to include water-soluble antibiotic polypeptides

encapsulated in polylactide/glycolide copolymers as well as similiar such polymers.

Microencapsulation for sustained release of enzymes, hormones, vaccines and other biologicals is discussed in a paper by Chang, Thomas, J. Bioeng., Vol 1, pp 25-32, 1976. Several examples of water-soluble protein encapsulations using polylactic acid are disclosed therein, particularly asparaginase and insulin compositions.

Polylactic acid polymers, polylactide/glycolide copolymers and polyglycolic acid polymers and related materials have been used for making surgical elements and the like, incorporating a medicament and demonstrating slow release properties. See for example U.S. Patents 3,991,776; 4,118,470; 4,076,798.

The compositions of this invention are hormonally active microcapsule formulations comprising at least one hormonally active polypeptide and optionally a polymer hydrolysis modifying agent intimately mixed with or coated by a biocompatable, biodegradable polymer which, when administered to a mammal, will release a daily amount of polypeptide effective for maintaining an hormonally related condition over a predetermined period of time.

The hormonally active polypeptides are luteinizing hormone-releasing hormone (LH-RH) polypeptides and analogues thereof. The specific hormonally related condition is the control of fertility and physiological effects related thereto.

One or more polymer hydrolysis modifying agents may optionally be present in these compositions. These agents, when present, may decrease or increase the rate of polymer hydrolysis. They are low molecular weight non-toxic organic acids, neutral or basic salts.

The encapsulating material is a synthetic polymer comprising certain poly(α-hydroxycarboxylic acids), poly(lactones), poly(acetals), poly(orthoesters) or poly(orthocarbonates).

The process for preparing these compositions is also disclosed, which process involves phase-separation techniques whereby the encapsulating polymer is precipitated onto water droplets containing the peptide and hydrolysis modifying agent, dispersed as an water-in-oil emulsion, by the addition of a coacervation agent which is a non-solvent for the encapsulating polymer. The capsules are then hardened, washed and dried.

Hormonally active polypeptides are those peptides which have a specific regulatory effect on the activity of a certain body organ as exemplified by those compounds secreted by the various endocrine glands or, additionally, compounds not secreted by an endocrine gland but having similiar activity.

The hormonally active polypeptides of this invention may be any of the polypeptides secreted by the endocrine glands, polypeptides not produced by a specific gland but having similair activity or analogues thereof. Of particular interest are the naturally occuring luteinizing hormone-releasing hormone (LH-RH) polypeptides and their synthetic analogues.

Naturally occuring LH-RH peptides are produced in the hypothalmic region of the brain and control the reproductive cycle of mammals by acting on the anterior pituitary gland to effect release of luteinizing homone (LH) and follicular stimulating hormone (FSH) which in turn act on the gonads to stimulate the synthesis of steroid hormones and to stimulate gamete maturation. The pulsatile release of LH-RH thereby controls the

reproductive cycle in mammals. Additionally, LH-RH has effects in placenta, in releasing HCG, and directly on the gonads. Agonist analogs of LH-RH are useful for the control of fertility by two mechanisms of action. Low doses of LH-RH analogs can stimulate ovulation and are useful in the treatment of hypothalmic and ovulatory infertility. Additionally they can be used for hypogonadal conditions and impotence, and to stimulate spermatogenesis and androgen production in the male. Paradoxically, larger doses of highly potent and long-lasting analogues of LH-RH have an opposite effect and block ovulation in the female and suppress spermatogenesis in the male. Related to these effects is a suppression of normal circulating levels of sexual steroids of gonadal origin, including reduction in accessory organ weight in the male and female. In domestic animals this paradoxical effect promotes weight gain in a feed-lot situation, stimulates abortion in pregnent animals and in general, acts as a chemical sterilant. A full list of the paradoxical high dose effects is set out in U.S. Patent application 47,661, filed June 11, 1979 and is incorporated herein by reference.

There is also the group of LH-RH analogues termed antagonists. These polypeptides have the paradoxical effect shown by LH-RH agonists but at low dose levels relative to naturally occuring LH-RH. Such compounds are to be included within the scope of this invention

The natural hormone releasing hormone LH-RH is a decapeptide comprised of naturally occuring amino acids (which have the L-configuration except for the achiral amino acid glycine). Its sequence is as follows: (pyro)Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2. Many analogues of this natural material have been studied. The benificial effectiveness of these analogs has been

varied. The most significant modification where agonists are concerned is obtained by changing the 6-position residue from Gly to a D-amino acid, for example, D-Ala, D-Leu, D-Phe or D-Trp. Antagonist activity can be best realized by substituting the naturally occuring 2-position His amino acid residue with with a D-amino acid residue. These analogues show increased activity relative to LH-RH.

In addition to modifications position 6, increased agonist activity may be obtained by the following modifications: modifying position 10 to afford a nonapeptide as an alkyl-, cycloalkyl- or fluoroalkyl-amine, or by replacing Gly-NH_2 by an α-azaglycine amide; substituting N-methyl-leucine for leucine in position 7; replacing tryptophan in position 3 by 3-(1-naphthyl)-L-alanine; substituting the position 5 tyrosine residue with phenylalanine or 3-(1-pentafluorophenyl)-L-alanine; and the subsititution at position 6 of unnatural D-amino acid residues containing two or more carbocyclic (or perhydroaryl) rings or a phenyl (or cyclohexyl) ring which is highly alkyl substituted. These specific compounds represent some of the more useful fertility affecting LH-RH type polypeptides which have been developed to date. This is not intended to be an exhaustive or exclusive list of all such compounds which which have been made or which can or may be made. They are simply set out to illustrate the type of compounds which are the subject of this invention. Any and all of them can be interchangeably substituted into the compositions of this invention.

The compounds of specific interest herein are those from the last mentioned group wherin the 6-position of the naturally occuring LH-RH material is replaced with a specific unnatural D-amino residue containing lipophilic

carbocyclic residues, particularly residues containing two or more highly alkyl substituted carbocyclic aryl (or perhydroaryl) rings or a phenyl (or cyclohexyl) ring. These particular polypeptides are the subject of U.S. Patent application No. 47,661, filed June 11, 1979 and are prepared in accordance with the procedures set forth therein. These polypeptides and there preparation are incorporated herein by reference.

More specifically the fertility affecting polypeptides of of particular interest in this invention are nonapeptides and decapeptides of the formula:

(pyro)Glu-His-V-Ser-W-X-Y-Arg-Pro-Z

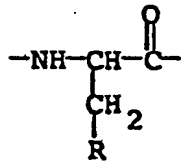
(I)

and the pharmaceutically acceptable salts there wherein:

V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;

W is tyrosyl, phenyalanyl or 3-(1-pentafluoro-phenyl)-L-alanyl;

X is a D-amino acid residue



wherein R is

(a) a carbocyclic aryl-containing radical selected from the group consisting of naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl and phenyl substituted with three or more straight chain lower alkyl

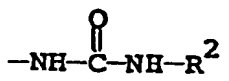
groups; or

(b) a saturated carbocyclic radical selected from the group consisting of cyclohexyl substituted with three or more straight chain lower alkyl groups, perhydronaphthyl, perhydrobiphenylyl, perhydro-2,2-diphenylmethyl and adamantyl;

Y is leucyl, Isoluecyl, nor-leucyl or N-methl-leucyl;

Z is glycinamide or $-NH-R_1$, wherein

R_1 is lower alkyl, cycloalkyl, fluoro lower alkyl or



R_2 is hydrogen or lower alkyl.

Preferred compounds of this invention are those wherein X is 3-(2-naphthyl)-D-alanyl or 3-(2,4,6-trimethylphenyl)-D-alanyl; Z is glycinamine; V is tryptophyl or phenylalanyl; W is tyrosyl and Y is leucyl or N-methyl-leucyl.

Particularly preferred compounds are:

(pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-Gly-NH_2,

(pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-n-methyl-Leu-Arg-Pro-Gly-NH_2,

(pyro)Glu-His-Phe-Ser-Tyr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-Gly-NH_2,

(pyro)Glu-His-Trp-Ser-Tyr-3-(2,4,6-trimethylphenyl)-D-alanyl-Leu-Arg-Pro-Gly-NH_2,

(pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphtyl)-D-alanyl-Leu-Arg-Pro-NHEt,

(pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphtyl)-D-alanyl-N-methyl-Leu-Arg-Pro-NHEt, and their pharmaceutically acceptable salts.

Especially preferred is (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-Gly-NH_2 and its pharmaceutically acceptable salts.

As set forth above and for convenience in describing these compounds, the conventional abbreviation for the various amino acids are used as generally accepted in the peptide art as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, Biochemistry, 11, 1726 (1972) and represent the L-amino acids with the exception of the achiral amino acids in the 6-position designated by X. All peptide sequences mentioned herein are written according to the generally accepted convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right. The abbreviation "Et" is monovalent ethane.

As used herein, the term "pharmaceutically acceptable salts" refer to the salts that retain the desired biological activity of the parent compound and do not impart any undesired toxicological effects. Examples of such salts can be found in U.S. Patent application 047,661, noted above, which is incorporated herein by reference.

As used herein the term "lower alkyl" refers to a straight or branched chain saturated hydrocarbon group having from 1 to 4 carbon atoms such as, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, and tert-butyl; the term "cycloalkyl group" refers to a cyclic saturated hydrocarbon group having from 3 to 6 carbon atoms, for example, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl; the term "fluoro lower alkyl" refers to a lower alkyl group wherein one or more hydrogen atoms are replaced by fluorine, such as, for example, trifluoromethyl, pentafluoroethyl, 2,2,2-trifluoroethyl, and the like.

As used herein "naphthyl" is inclusive of 1- and 2-naphthyl; "anthryl" is inclusive of 1-, 2- and 9-anthryl; "fluoroenyl" is inclusive of 2-, 3-, 4-, and 9-fluoroenyl; "phenanthryl" is inclusive of 2-, 3- and

9-phenanthryl; and "adamantyl" is inclusive of 1- and 2-adamantyl.

As used herein the phrase "fertility affecting polypeptide" should be understood to mean any naturally occuring LH-RH polypeptide, synthetically prepared material of the same type or synthetically prepared analogues of naturally occuring LH-RH polypeptides which act in some manner on the anterior pituitary gland to effect the release of luteinizing hormone (LH) and follicular stimulating hormone (FSH); and in particular those polypeptides which inhibit ovulation or are useful for treating endometriosis in a female mammalian subject or are useful for treating benign prostatic hypertrophy and inhibiting spermatogenesis in a male mammalian subject.

The compositions of this invention will contain the hormonally active polypeptides in varying amounts depending upon the effect desired. Treatment of infertility requires a low level of drug, while prevention of fertility and related effects requires a large dose relative to the activity of naturally occuring LH-RH. For the agonist fertility control it is expedient to prepare microcapsules which will release the drug at such a rate that the subject will receive between about 0.01 and 100 µg/kg body weight per day, preferably between 0.1 and 5.0 µg/kg body weight per day.

The compositions of this invention are formulated to contain the polypeptide in an amount which may vary between 0.01 and 40.0 weight % of the polymer used for encapsulation. Preferably the peptide will be present in the amount between 0.1 to 10.0 weight %.

The amount of drug placed in a particular formulation depends not only on the desired daily dose but also on the number of days that dose level is to be maintained. While this amount can be calculated

empirically the actual dose delivered is a function of the degradation characteristics of the encapsulating polymer. Therefore the % weight of drug stated represent amounts which, when taken in conjunction with a particular polymer provide the desired release profile.

Optionally, certain chemicals which affect the rate of polymer hydrolysis may be dissolved in the aqueous solution containing the polypeptide before it is encapsulated by the polymer excipient. These chemicals are called polymer hydrolysis modifying agents. When present, these compounds may increase or decrease the rate at which the drug is released from the microcapsules. This affect is independent of a particular polymer composition or size.

Four types of chemicals may be used to realize this effect, for example, organic acids, acidic neutral or basic salts. Low molecular weight mono and dicarboxylic acids such as acetic acid, tartaric acid, citric acid, gluconic acid, oxalic acid, ascorbic acid, succinic acid, their salts, and the like may be used. Basic salts may be, for example, ammonium sulfate, ammonium chloride, ammonium nitrate, sodium bisulphate and the like. Neutral salts effective herein include metal halides such as, for example, sodium chloride, potassium chloride, sodium bromide, potassuim bromide, calcium chloride, magnesium chloride and the like. Basic salts include such salts as sodium carbonate, potassuim carbonate, trisodium phosphate, tripotassium phosphate and the like. Of these compounds it is most preferred to use either citric acid, sodium chloride or sodium carbonate. Combinations of these compounds will achieve the desired affect but the compositions described herein contain only one of these agents in a particular composition.

When present the hydrolysis modifying agent will be added in an amount between 0.1 and 20% by weight of the

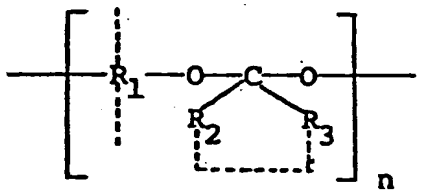
polymer but preferably it will be present in the amount of 5 to 10%.

The number and type of encapsulating excipients which may be effectively used to practice this invention is limited only by the requirements that the material be biocompatable and biodegradable. That is, the polymer must be non-toxic to the host and must be of such composition that it is degradable by the body into metabolic products that have no deleterious or untoward effects on the body. These polymers must also be capable of forming microcapsules containing water-soluble drugs.

A number of polmers have been developed which meet these criteria. Various combinations of alpha hydroxy-carboxylic acids and certain lactones can be condensed to form such polymers, particularly lactic acid and glycolic acid or combinations thereof. See, for example U.S. Patent No. 3,773, 919. Similiar biocompatable polymers based on glycolic acid and glycerol and the like also are known. See U.S. Patents 3,991,776; 4,076,779 and 4,118,470 for examples of such compositions. Several new biocompatable, biodegradable polymers derived from polyorthoesters and polyorthocarbonates also may be effectively used as encapsulating excipients in the practice of this invention. These latter polymers are described in U.S. Patents 4,093,709 and 4,138,344. There are also known polyacetals and polyorthoesters useful for this purpose as described in Polymer Letters 18, 293 (1980). This list is not intended to be exhaustive of the polymers which are compatable with the scope and intention of this invention but merely sets out examples to illustrate the type of polymer excipients which may be used.

One preferred group of polymer excipients are the orthoester and orthocarbonate polymers having a repeating mer comprising a hydrocarbon radical and a symmetrical

dioxycarbon unit of the general formula:



wherein R_1 is a multivalent hydrocarbon radical, R_2 and R_3 are hydrocarbon radicals with at least one of R_2 or R_3 bonded to the dioxycarbon through the oxygen linkage, and which polymers are synthesized by reacting a polyol with an orthoester or orthocarbonate. A full and complete description of the exact compositions, preparation, and properties of these polymers can be found in U.S. Patents 4,093,709 and 4,138,344, which are incorporated by reference as if fully set out herein.

Also preferred are those polymers based on the condensation of divinyl ethers and polyols. These compounds are prepared by reacting polyol with a diketene acetal to form the polyacetal. A more detailed description and discussion of these polymers can be found in the journal, Polymer Letters, J. Heller, et al, 18, 293 (1980), which is incorporated herein by reference. Of similiar interest are those polyorthoesters prepared by a modification of the synthesis used to prepare the above polyacetals. These polymers are comprised of diketene acetal-diol condensates. For example, the diketene acetal 3,9-bis-(methylene)-2,4,-8,10-tetr-aoxaspiro[5,5]undecane can be condensed with 1,6-hexanediol to give a polyorthoester polymer which has degradation properties in vivo which make its use in the compositions of this invention desirable. Further preparation techniques and polymer characteristics for these compounds can be found in U.S.Patent Nos.

4,093,709; 4,131,648; 4,138,344; and 4,180,646 all of which are incorporated herein by reference.

Most preferred herein are those polymers derived from the condensation of alpha hydroxycarboxylic acids and related lactones. The most preferred polymer excipients herein are derived from an alpha hydroxy acid, particularly lactic acid, glycolic acid or a mixture of the two.

The alpha hydroxy acid units from which the preferred excipients are prepared may be the optically active (D- and L-) forms or optically inactive (DL-, racemic) forms. For example, lactic acid, whether it is the principle polymer component or the comonomer component, can be present as D-lactic acid, L-lactic acid or DL-lactic acid.

Other comonomers, for example certain C3 to C18 carboxylic acids and certain lactones, can be used in the preparation of preferred polymers. Illustrative of such compounds are 3-propiolactone, tetramethylglycolide, b-butyrolactone, 4-butyrolactone, pivalolactone, and intermolecular cyclid esters of α-hydroxy butyric acid, α-hydroxyisobutyric acid, α-hydroxyvaleric acid, α-hydroxyisovaleric acid, α-hydroxy caproic acid, α-hydroxy-α-ethylbutyric acid,, α-hydroxyisopcaproic acid, α-hydroxy-3-methylvaleric acid, α-hydroxy-heptanoic acid, α-hydroxyoctanoic acid, α-hydroxydecanoic acid, α-hydroxymysristic acid, α-hydroxystearic acid, and α-hydroxylignoceric acid.

Any of these compounds may be used a comonomer in the preparation of acceptable polymers. 3-butyrolactone can be used as the sole monomer or as the principle monomer along with any of the comonomers recited above. However it is most preferred to use lactic acid as the sole monomer or lactic acid as the principle monomer with glycolic acid as the comonomer.

The term polylactide is used to designate the general class of polymers which can be prepared from one or more of the preferred monomers listed above and includes those instances where a single alpha hydroxy acid or lactone is the only monomer in the polymer. For the most preferred polymers, those wherein the excipients are prepared solely from the lactic acid monomer or where lactic acid is the principle monomer and glycolic acid is the comonomer are termed poly(lactide-co-glycolide) copolymers.

The combinations of prefered monomer and comonomer which can be prepared are numerous but the most effective excipients are those polymers prepared from lactic acid alone or lactic acid and glycolic acid wherein the glycolic acid is present as a comonomer in a molar ratio of 100:0 to 40:60. It is most preferred to use a poly(lactide-co-glycolide) copolymer having a molar ratio between about 75:25 and 50:50.

Poly(lactide-co-glycolide) polymers may range in size from about 20,000 to about 100,000 in molecular weight, stated as an average. The molecular weight of a particular copolymer is independent of its monomeric makeup. For example, a 50:50 copolymer can have a molecular weight which falls anywhere within this range. Therefore polymers can be varied both as to their monomer composition and as well as their molecular weight and be within the scope and intent of this invention.

For the purposes of this invention the relative molecular weight of a particular polymer vis-a-vis a second polymer is stated in terms of its inherent viscosity in a particular solvent and at a particular temperature. The viscosity of a particular polymer is measured in a capillary viscometer using chloroform or hexafluoroisopropanol at 30°C. The results are stated in terms of deciliters/g (dl/g). There is a direct

correlation between inherent viscosity and molecular weight.

A method for the preparation of polylactide polymers can be found in U.S. Patent 3,773,919 and reference is made thereto for the preparation of the such polymers which is incorporated herein by reference.

Preparation of the microcapsules using any combination of the various peptides, polymer hydrolysis modifying agents or encapsulating polymer excipients noted above parallels the basic technique set out in U.S. Patent 3,773,919. A full description of the procedure used herein can be found in that document.

In brief, the procedure involves dissolving the polymer in an halogenated hydrocarbon solvent, dispersing the aqueous drug solution in this polymer-solvent solution, and adding some agent which is soluble in the halogenated hydrocarbon solvent but is a non-solvent for the encapsulating excipient. The addition of the non-solvent, called a coacervation agent, causes the excipient to precipitate out of the halogenated hydrocarbon solvent onto the dispersed water droplets, thereby encapsulating the polypeptide. For example, a poly(lactide-co-glycolide) is dissolved in methylene chloride. An aqueous solution of polypeptide is then stirred into the solvent-polymer solution to form an water-in-oil emulsion. A second solvent-miscible material such as a silicone oil, is added slowly with stirring to precipitate the excipient which coats the dispersed water droplets to give microcapsules.

Halogenated organic solvents which may be used are most of the C1 to C4 halogenated alkanes such as, for example, methylene chloride, ethylene dichloride, ethylene chloride, 2,2,2-trichloroethane and the like.

Coacervation agents may be any solvent miscible polymeric, mineral oil or vegetable oil compounds which

are non-solvents for the encapsulating polymers. There may be used, for example, silicone oil, peanut oil, soybean oil, corn oil, cotton seed oil, coconut oil, linseed oil, mineral oils and other related oils.

After being formed, the microcapsules are washed and hardened with an alkane organic solvent, washed with water, washed with an aqueous non-ionic surfactant solution, and then dried at room temperature under vacuum.

Microcapsules may range in diameter from about 1 to 500 microns, depending upon the techniques empoloyed. For this invention it is preferred to have the microcapsule diameter be between 5 and 200 microns.

The prepared microcapsules may be administered to a subject by any means or route desired. However the most effacious route is parenteral administration by injection, most preferably subcutaneously or intramuscularly.

If the capsules are to be administered by injection they may first be suspended in some non-toxic suspending vehicle. The exact make up of these injectable microcapsule suspensions will depend upon the amount of drug to be administered, the suspending capacity of the suspending agent and on the volume of solution which can be injected at a particular site or in a particular subject.

The compositions of this invention exhibit sustained release of the encapsulated compounds over extended periods of time. This time period may range from one month to 3 years depending on the composition of the encapsulating excipient, its molecular weight, the diameter of the capsule, and the presence of a polymer hydrolysis modifying agent in the core. Preferably the release time will be about 1 to 24 months.

The following examples illustrate the compositions and processes of this invention.

Example I

This example describes the procedure for preparing a microcapsules composition wherein the polypeptide is (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-Gly-NH_2, (D-Nal(2)^6 LH-RH) present in an amount of 1.4% by weight, no polymer hydrolysis modifying agent is present, and the excipient is a 50:50% molar ratio poly(lactide-co-glycolide) copolymer having an inherent viscosity in hexafluoroisopropanol of 0.38 dl/g at 30°C.

Excipient, 4 g, were dissolved in 196 g of methylene chloride. This solution was placed in a 300 ml resin kettle equipped with a true-bore stirrer having a 2.5 inch Teflon turbine impeller driven by a Fisher "Stedi-Speed" motor. In a 1-dram glass vial was dissolved 0.0571 g of polypeptide in 1.34 g of deionized water. This solution was added to the resin kettle. During this addition, the dilute polymer solution was stirred at 3200 RPM to form a water-in-oil emulsion. With continued stirring at that rate, 80 ml of silicone oil was added at the rate of 4.0 ml/min by means of a peristaltic pump. The silicone oil caused the polymer to phase separate, and deposit as droplets of solvent-swollen polymer onto the surface of the water-polypeptide microdroplets. These solvent-swollen polymer droplets then coalesced to form a continuous film around the water-polypeptide microdroplets. The microcapsules were then hardened by pouring the contents of the resin kettle into a beaker containing 2000 ml of heptane. This mixture was stirred at 1000 RPM for 30 minutes with a stainless-steel impeller. The heptane-methylene chloride-silicone oil solution was removed by filtering the solution, employing

a Buchner funnel and Whatman β41 filter paper. The microcapsules were then washed repeatedly with 100-ml aliquots of heptane to insure complete removal of the silicone oil. The microcapsules were then washed with deionized water followed by a wash with a 1% aqueous solution of Tween 20. and dried at room temperature under vacuum. Microcapsules obtained from this preparation were determined to have diameters ranging in size from 10 to 40 microns.

The polypeptide containing microcapsules, whose preparation is described in the above paragragh, were suspended in a suspending vehicle and administered as a single subcutaneous injection to female Sprague-Dawley rats and female rhesus monkeys. The length of estrous suppression was calculated against the percentage of animals showing suppression.

The results of the monkey study are given in Table I below. Each data line represents one subject. The injected dose was as stated in the Table. Microcapsules were prepared as stated in Example I using that LH-RH analogue and a 50:50 % molar ratio copolymer (PLA:PGA) having an inherent viscosity of 0.38 dl/g in hexafluoro-isopropanol at 30°C at a 1.4% peptide to polymer ratio. The microcapsule's diameter ranged from 10 to 40 μm.

TABLE I

EFFECT OF D-Nal(2)^6 LHRH RELEASED FROM PLA:PGA MICROSPHERES ON OVULATION IN RHESUS MONKEYS

ANIMAL NO.	DOSE	INTERMENSTRUAL INTERVAL BEFORE	DURING	AFTER TREATMENT
1	--	25	30	28
2	--	28	27	26, 29
3	1mg D-Nal(2)^6	30	67	27
4	1mg D-Nal(2)^6	24	83	27

A single 300 µg dose of D-Nal(2)^6 LH-RH micro-encapsulated at 1.4 % peptide to polymer with a 50:50 % molar ratio poly(lactide-co-glycolide) having a diameter ranging in size from 10-40 µm (inherent viscosity in hexafluoroisopropanol-0.38 dl/g) which had been suspended in a suspending agent (composition given in Example III) was injected subcutaneously in 10 mature female Sprague-Dawley rats. Estrous was determined by daily vaginal smear analysis. All rats showed estrous suppression through day 24 post dosing. At day 25, 40% showed estrous. By day 27 estrous was observed in all animals.

Example II

Table II sets out several examples of polypeptide containing microcapsules wherein the following parameters were varied: lactide-glycolide mole ratio; molecular weight, stated as inherent viscosity; stir rate; addition rate of silicone oil; and the amount of silicone oil added. The polypeptide encapsulated here is the same as set out in Example I. The preparation techniques described in Example I were used to prepare these materials, except as note for the stirring rates and silicone oil addition rates.

TABLE II

Batch	Excipient's Inherent Viscosity, dl/g	Lactide:Glycolide Mole Ratio	Polymer (g)	Peptide (g)	Silicone Oil Am't Added (ml)	Silicone Oil Rate Added (ml/min)	Stir Rate RPM	Capsule Size µm
A	0.47[2]	75:25	2.0	0.0266	40.0	2.0	1000	40.5% 45µm 44.4% 45µm
B	0.97[2]	68:32	2.0	0.0255	40.0	4.0	3600	14.0% 45µm 77.0% 45µm
C	0.38[1]	50:50	2.0	0.0263	40.0	4.0	3000	10-30
D	0.38[1]	50:50	2.0	0.0279	40.0	4.4	3000	8-25
E	0.38[1]	50:50	2.0	0.0297	135.0	2.0	1000	45-90
F	1.52[1]	50:50	2.0	0.0253	40.0	4.0	3000	80-160

1 Inherent viscosity in hexafluoroisopropanol at 30°C.

2 Inherent viscosity in chloroform at 30°C.

In each of the above batches the following solvents and amounts used:

to dissolve the peptide - 0.67 ml of deionized water;

encapsulation solution - 98 ml of methylene chloride.

Example III

The following describes a formulation for parenteral injection of polypeptide-containing microcapsules prepared according to the methods disclosed herein.

Microcapsules containing the polypeptide (pyro)Glu-His-Trp-Ser-Tyr-3-(naphthyl)-D-alanyl-leu-Arg-Pro-Gly-NH_2 in a concentration of 1.0 % by weight and wherein the excipient polymer was poly(lactide-co-glycolide) having a molar ratio of 50:50 % and an inherent viscosity of 0.38 dl/g in hexafluoroisopropanol at 30°C were suspended in the following solution:

Na CMC	0.5%
NaCl	0.8%
Benzyl alcohol	0.9%
Tween 80	0.1%
Purified water	q.s. 100%

For example, 330 mg of microcapsules were suspended in 5.5 ml to provide an injectable dose of 300 µg of peptide per 0.5 ml of injectable suspension.

The foregoing discussion and specific embodiments are intended to be exemplary as to the scope and practice of this invention and should not be read to limit the practice of the art described therein.

CLAIMS:

1. A pharmaceutical composition designed for sustained release of an effective amount of drug over an extended period of time prepared in microcapsule form wherein the composition comprises:

at least one hormonally active polypeptide in an effective amount greater then a conventional single dose;

optionally, at least one polymer hydrolysis modifying agent; and

a biocompatable, biodegradable encapsulating polymer.

2. A composition of Claim 1 wherein said polypeptide is a luteinizing hormone-releasing hormone or an analogue thereof;

said hydrolysis modifying agent, if present, is an organic acid, acid salt, neutral salt or basic salt; and

said polymer is a polylactide polymer, polyacetal polymer, polyorthoester polymer or polyorthocarbonate polymer.

3. A composition of Claim 1 wherein said polypeptide is a nonapeptide or a decapeptide analogue of LH-RH having the formula

(pyro)Glu-His-V-Ser-W-X-Y-Arg-Pro-Z

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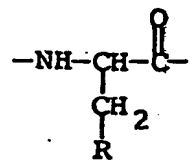
and the pharmaceutically acceptable salts thereof wherein:

V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;

W is tyrosyl, phenylalanyl or 3-(1-pentafluorophenyl)-L-alanyl;

X is a D-amino acid residue



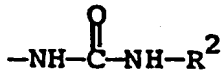
wherein R is

(a) a carbocyclic aryl-containing radical selected from naphthyl, anthryl, fluorenyl, phenylanthryl, biphenylyl, benzhydryl and phenyl substituted with three or more straight chain C_{1-4} alkyl groups; or

(b) a saturated carbocyclic radical selected from cyclohexyl substituted with three or more straight chain C_{1-4} alkyl groups, perhydronaphthyl, perhydrobiphenylyl, perhydro-2,2-diphenylmethyl and adamantyl;

Y is leucyl, isoleucyl, nor-leucyl or N-methyl-leucyl;

Z is glycinamide or $-NH-R_1$, wherein R_1 is C_{1-4} alkyl, C_{3-6} cycloalkyl, fluoro C_{1-4} alkyl or



R_2 is hydrogen or C_{1-4} alkyl.

4. A composition of Claim 3 having a polymer which is a poly(lactide-co-glycolide) copolymer wherein the copolymer comprises lactide-glycolide in a molar ratio of between 100:0 and 40:60 ; and wherein the copolymer has an average molecular weight between about 20,000 and 100,000.

5. A composition of Claim 4 wherein said polypeptide is present in an amount of between 0.01 and 40.0 weight % of the polymer; and said hydrolysis modifying agent is present in an amount of between 1 and 15 weight % of the polymer.

6. A composition of Claim 4 or Claim 5 having a polypeptide wherein:
V is tryptophyl or phenylalanyl;
W is tyrosyl;
X is 3-(2-naphthyl)-D-alanyl or 3-(2,4,6-trimethyl-phenyl)-D-alanyl;
Y is leucyl or N-methyl-leucyl; and
Z is glycinamide or NHEt;
said hydrolysis modifying agent is citric acid, ammonium chloride sodium chloride or sodium carbonate; and
said polymer comprises lactide-co-glycolide in a molar ratio of between 75:25 and 50:50.

7. A composition of Claim 6 wherein said polypeptide is present in an amount of 0.1 to 10.0 weight %;
said hydrolysis modifying agent is present an an amount of 5 to 10 weight %; and
said polymer is present in the molar ratio of 50:50.

8. A composition of Claim 7 wherein said peptide is (pyro)Glu-His-Trp-Ser-Tyr-3-(naphthyl)-D-alanyl-leu-Arg-Pro-Gly-NH_2 or a pharmaceutically acceptable acid salt thereof.

9. A composition of any one of the preceding Claims in the form of injectable particles ranging in size from about 0.1 to 500 microns.

10. A composition of any of Claims 1 to 9 which are dispersed in a pharmaceutically acceptable carrier suitable for parenteral administration.

11. A process for preparing a composition of Claim 1 comprising:

dispersing an aqueous solution containing the polypeptide, an optionally a polymer hydrolysis modifying agent, in a halogenated organic solvent containing said encapsulating polymer;
adding to the dispersion a coacervation agent;
hardening the microcapsules;
collecting the microcapcules from this solution;
washing the microcapsules; and
drying the microcapsules.

CLAIMS:

1. A process for preparing a pharmaceutical composition designed for sustained release of an effective amount of drug over an extended period of time prepared in microcapsule form; characterised in that the process comprises:

dispersing an aqueous solution containing a hormonally active polypeptide, and optionally a polymer hydrolysis modifying agent, in a halogenated organic solvent containing a biocompatable, biodegradable encapsulating polymer;
adding to the dispersion a coacervation agent;
hardening the microcapsules;
collecting the microcapsules from this solution;
washing the microcapsules; and
drying the microcapsules.

2. A process of Claim 1 wherein
said polypeptide is a luteinizing hormone-releasing hormone or an analogue thereof;
said hydrolysis modifying agent, if present, is an organic acid, acid salt, neutral salt or basic salt; and
said polymer is a polylactide polymer, polyacetal polymer, polyorthoester polymer or polyorthocarbonate polymer.

3. A process of Claim 1 wherein said polypeptide is a nonapeptide or a decapeptide analogue of LH-RH having the formula

(pyro)Glu-His-V-Ser-W-X-Y-Arg-Pro-Z

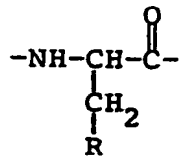
I

and the pharmaceutically acceptable salts thereof wherein:

V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;

W is tyrosyl, phenylalanyl or 3-(1-pentafluorophenyl)-L-alanyl;

X is a D-amino acid residue



wherein R is

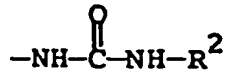
(a) a carbocyclic aryl-containing radical selected from naphthyl, anthryl, fluorenyl, phenylanthryl, biphenylyl, benzhydryl and phenyl substituted with three or more straight chain C_{1-4} alkyl groups; or

(b) a saturated carbocyclic radical selected from cyclohexyl substituted with three or more straight chain C_{1-4} alkyl groups, perhydronaphthyl, perhydrobiphenylyl, perhydro-2,2-diphenylmethyl and adamantyl;

Y is leucyl, isoleucyl, nor-leucyl or N-methyl-leucyl;

Z is glycinamide or $-NH-R_1$, wherein

R_1 is C_{1-4} alkyl, C_{3-6} cycloalkyl, fluoro C_{1-4} alkyl or



R_2 is hydrogen or C_{1-4} alkyl.

4. A process of Claim 3 using a polymer which is a poly(lactide-co-glycolide) copolymer wherein the copolymer comprises lactide-glycolide in a molar ratio of between 100:0 and 40:60 ; and wherein the copolymer has an average molecular weight between about 20,000 and 100,000.

5. A process of Claim 4 wherein said polypeptide is present in an amount of between 0.01 and 40.0 weight % of the polymer; and said hydrolysis modifying agent is present in an amount of between 1 and 15 weight % of the polymer.

6. A process of Claim 4 or Claim 5 using a polypeptide wherein:

V is tryptophyl or phenylalanyl;
W is tyrosyl;
X is 3-(2-naphthyl)-D-alanyl or 3-(2,4,6-trimethyl-phenyl)-D-alanyl;
Y is leucyl or N-methyl-leucyl; and
Z is glycinamide or NHEt;
said hydrolysis modifying agent is citric acid, ammonium chloride sodium chloride or sodium carbonate; and
said polymer comprises lactide-co-glycolide in a molar ratio of between 75:25 and 50:50.

7. A process of Claim 6 wherein said polypeptide is present in an amount of 0.1 to 10.0 weight %;
said hydrolysis modifying agent is present an an amount of 5 to 10 weight %; and
said polymer is present in the molar ratio of 50:50.

8. A process of Claim 7 wherein said peptide is (pyro)Glu-His-Trp-Ser-Tyr-3-(naphthyl)-D-alanyl-leu-Arg-Pro-Gly-NH_2 or a pharmaceutically acceptable acid salt thereof.

9. A process of any one of the preceding claims wherein the product is in the form of injectable particles ranging in size from about 0.1 to 500 microns.

10. A process of any one of the preceding claims wherein the product composition is dispersed in a pharmaceutically acceptable carrier suitable for parenteral administration.